

REMARKS

This Preliminary Amendment is filed concurrently with filing the Request for Continuing Examination and in response to the Examiner's Advisory Action dated January 28, 2010.

Status of the Claims

Claims 85-88, 90, 91 and 93-97 are pending. Claim 97 is added. Claims 85, 89, and 92 are canceled.

Claims 87, 91, 93, 95 and 96 are amended. Support for amendment of claims is found in [0215], [0252] and throughout the specification.

The new claim 97 is supported in paragraphs [0078], [0074], [0113], [0115], [0124], [0193], [0208] and elsewhere in the specification.

Claim Rejections under 35 USC § 112

Claims 85-96 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Examiner argues that support is not found in the specification for at least 125% more S-GAG (bridging lines 26 and 27 of claim 85).

Applicants disagree. Claim 85 does not seem to contain the language Examiner is referring to. If Examiner meant claim 96, claim 96 contain the following language:

“wherein production of S-GAG by said activated chondrocytes is increased to at least 152% compared to chondrocytes not subjected to said activation and wherein a DNA content index, determined by Hoechst Dye DNA assay, is increased by said activated chondrocytes to the DNA content index 1.49 compared to that of the DNA content index 1 observed in chondrocytes not subjected to said activation”.

Examiner agrees that the 152% increase is disclosed in paragraph 228. Applicants amended claim 96 to read “wherein production of S-GAG by said activated chondrocytes is increased to 152%”. The language “up to” is canceled although Applicants maintain that it is warranted by results disclosed in the specification with regard to specific pressures, flow rates and CO₂ concentration as described in Tables 2, 3 and 4.

With this amendment the prior rejections under 35USC112, first paragraph is overcome.

Claim Rejections - 35 USC § 112

Claims 87 and 91 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claim 87, "the space" does not have clear antecedent basis.

Applicants disagree, however, to expedite the prosecution, Applicants canceled the objected to language from claim 87.

In claim 91, "Tissue Engineering Support system" is a trade mark representing a specific system. The components of this specific system and how it works in the method of the claims is unclear.

Applicants disagree. The TESS is disclosed in the specification, however, to expedite the prosecution applicants amended claim 91 to use more descriptive language concerning the tissue processor. The tissue processor works as claimed in claim 96, that is an activation of chondrocytes is performed in a tissue processor and comprises treating the matrix seeded with said chondrocytes with a cyclic or constant hydrostatic pressure about 0.5 MPa above atmospheric pressure, under perfusion with a perfusion medium at a rate of perfusion flow about 5 μ L /minute and under oxygen concentration about 2-5%. The tissue processor, as claimed in claim 91, is disclosed in paragraph [0177].

Applicants submit that with these amendments, the rejections under 35USC 112, second paragraph, is overcome.

Claim Rejections - 35 USC § 102

Claims 85-96 are rejected under 35 U.S.C. 102(a) as being anticipated by Smith, et al (6,528,052).

Examiner argues that the claims are drawn to a hyaline cartilage construct produced by a method of preparing a collagenous porous support matrix having pores between 100 and 300 μ m, seeding the support with chondrocytes activated in a tissue processor under conditions of cyclic or constant hydrostatic pressure so the chondrocytes synthesize an extracellular matrix, S-GAG and DNA, and at least 152% more of S-GAG is produced, and DNA is increased to an content index 1.49. Activation involves applying a cyclic or constant hydrostatic pressure from about 0.5 to 5 MPa above atmospheric pressure at a frequency of from about 0.01 to 2 Hz for about one hour to 30 days followed by a resting period from

about one day to sixty days, under perfusion with a perfusion medium at a flow rate from about 1 to 500 μL per minute under an oxygen concentration of 1-20%.

Applicants basically agree with this assessment of previously pending claims. However, in order to speed up the prosecution, Applicants amended claims introducing further limitations.

Examiner argues that Smith et al disclose repair and regeneration of cartilage by a process that involves *in vivo*, *ex vivo* or *in vitro* treatment of cartilage or cartilage cells (chondrocytes) in a support such as a scaffold or collagen matrix (col 6, lines 14-16) by using a loading regimen involving conditions of intermittent application of periods of hydrostatic pressure followed by periods of recovery *in situ* (col 4, lines 25-31, and col 7, line 30 to col 8, line 8). The recovery period can be at atmospheric or low constant pressure (col 7, lines 48-50). *In vitro* treatment is performed by obtaining cartilage cells from cartilage, and applying the loading regimen conditions while culturing the cartilage cells in suspension within a scaffold/support, and implanting the resultant tissue or cells into a patient (col 9, lines 23-30, and col 11, lines 5-9). Articular chondrocytes (col 16, line 65) are isolated from cartilage using enzyme digestion (col 17, line 4). The chondrocytes can be autologous or not autologous (col 9, line 33). Articular cartilage can be regenerated and repaired (col 1, lines 41-43).

Examiner maintains that a cartilage construct produced by the process of Smith et al is the same the construct presently claimed for implantation into a cartilage lesion or defect. No difference is seen in the presently claimed process from the process of Smith et al that would result in a materially different construct. The process of Smith et al will inherently produce a construct containing extracellular matrix, S-GAG and DNA as claimed.

Applicants disagree. Examiner is relying on the elusive doctrine of the patent law, namely on the inherent anticipation. It is Applicants position that the Smith et al invention and the instant construct are not the same and if both methods were used alongside each other, the instant invention would be superior in its properties to that of Smith et al because of the presence of activated chondrocytes according to the invention. Moreover, Applicants added claim 97 directed to a construct that is not a product by process claim.

As had been previously argued by Applicant and ignored by Examiner, the instant product by process claims are directed to an implantable hyaline cartilage construct having properties not disclosed and not present in the Smith reference disclosing a method for repair and regeneration of cartilage.

The instant construct consist of two components, namely a collagenous support matrix and activated chondrocytes, each having properties not disclosed by Smith and therefore not enabled by Smith.

A collagenous support matrix has pores limited to 100-300 μm . Importance of the pore size is disclosed in [0153]. The limited pore size of the support matrix controls infiltration of chondrocytes into the construct and growth and propagation of cells within the construct. Such growth and propagation result in unexpectedly high production of S-GAG. Neither pore size and/or its importance for chondrocytes growth and propagation or extraordinarily high production of S-GAG is disclosed by Smith. As a matter of facts, Smith discloses and exemplarize use of cell monolayers (17:21-27; 17:55-62; and 18:30-36) and use of grafts removed from osteoarthritic joints (4:55-65; and 7:9-17).

Activated chondrocytes of the invention are chondrocytes that were infiltrated into the support matrix having limited pore size and therefore their growth and propagation proceeded differently from the treatment of Smith cell monolayers or grafts.

The activation process consists of some, but not all, steps used by Smith. For example, the instant activation is shown to result in up to 152% increase of S-GAG for hydrostatic pressure and even higher levels for cyclic pressure, indicating a production of extracellular matrix by dividing and multiplying activated chondrocytes that were shown to promote growth of large amounts of an extracellular matrix. Production of S-GAG is not disclosed or recognized by Smith. Smith is dealing with cell monolayers and or with grafts of tissue removed from diseased joints.

The activation process according to the invention is performed in the tissue processor that has very controllable conditions and the process does not include only the application of hydrostatic pressure but includes also perfusion under certain perfusion flow and medium and in the presence of oxygen and carbon dioxide gases. Each of these conditions produces increased amount of S-GAG. Smith does not disclose, describe or imply use of any of these conditions. Smith utilizes solely the apparatus for intermittent hydrostatic loading.

Examiner argues that the construct of the invention is inherently anticipating the instant claims and that the cells of Smith would perform the same. Applicants disagree. Applicants provide ample evidence that the pore size of the matrix, perfusion flow rate, presence or absence of oxygen and carbon dioxide and the timing for the activation are of importance and results in production of high levels of S-GAG and DNA. The instant activated cells within the matrix by the instantly claimed process including all conditions are

therefore different in their performance from the cells submitted just to the hydrostatic pressure.

Applicants maintain that the construct, produced by activation of chondrocytes and containing high level of extracellular matrix macromolecules produced due to a complex condition to which are the chondrocytes subjected during activation as a whole, is not produced by Smith reference.

The activated chondrocytes are in an active stage where they divide, multiply and promote growth of the extracellular matrix by accumulating extracellular macromolecules, such as sulfated glycosaminoglycan (S-GAG), and DNA, to the extent that is the subject matter claimed herein, are nowhere disclosed in Smith.

Conditions that Examiner dismisses as not being important for distinguishing the invention from that of Smith reference, account for increased levels of production of S-GAG and therefore increased production of extracellular matter and for increased levels of DNA content index resulting in increased cell proliferation. Only due to the instant process, the cell proliferation can be increased or decreased and the production of S-GAG can be regulated. Therefore, the instant invention, i.e., the construct fabricated under the preset set of conditions according to the invention process, results in substantially increased production of S-GAG and DNA by more than 50% (152%) and 49% (DNA index increase to 1.49), respectively. The yield that can be manipulated and particularly such high yields as disclosed herein are nowhere disclosed in Smith et al.

Anticipation, and even implied anticipation, requires that the anticipatory and instant inventions are the same, that is that the construct, or the process for its production are the same, as well as that the construct of the prior art and the construct of the invention functions in the substantially the same way with the substantially the same results. Quite clearly that is not so. The Smith method is not the same, does not produce the same activated chondrocytes and nowhere discloses the results in S-GAG production and DNA increase. A reason is that Smith does not use controlled conditions, such as presence of low concentrations of oxygen or carbon dioxide, low perfusion rate, limited pore size for the scaffold, cell loading density or use of tissue processor that were shown to be instrumental in the functioning construct of the invention able to synthesize unexpectedly high amounts of extracellular matrix molecules. Activated chondrocytes according to the instant invention are not the same as chondrocytes of Smith subjected solely to the intermittent hydrostatic pressure.

It is submitted that the invention is not anticipated by Smith et al and the rejections should be withdrawn. It is respectfully requested that Examiner withdraws the rejections under 35 USC 102 over Smith et al.

Claim Rejections - 35 USC § 103

Claims 85-96 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith et al (6,528,052) in view of Lee et al (6,306,169) and Burg (6,991,652), and if necessary in further view of Atkinson et al (6,511,958).

The invention and Smith et al are described above.

Examiner argues that Lee et al disclose producing an implant containing cells such as chondrocytes (col 7, line 8) by isolating the cells from tissue, proliferating the cells in a medium containing serum to obtain a sufficient number of cells, and seeding the cells in a construct (col 7, lines 13-17) such as a collagen sponge (col 12, line 17). A collagen sponge can be infiltrated with an alginate or agarose solution containing the cells, and the alginate or agarose gelled within the sponge (col 13, lines 11-25). This procedure produces a construct having mechanical function that resembles that processed by tissue to be repaired (col 4, lines 28-37).

Examiner further argues that Burg discloses forming a hydrogel-cell composition for use in forming new tissue such as cartilage. Before the cell are incorporated in a construct, the cells can be expanded in number by culturing *in vitro* in a medium containing serum (col 7, lines 20-29). Temperature-dependent hydrogels can be used (paragraph bridging cols 5 and 6). The hydrogels have reverse gelation properties, and are liquids at or below room temperature, and gel when warmed to higher temperatures, e.g. body temperature.

Examiner concludes that when incorporating chondrocytes from cartilage into a scaffold for treatment as disclosed by Smith et al, it would have been obvious to expand the number of cells by *in vitro* culturing in a culture medium prior to incorporating the cells in the scaffold as suggested by Lee et al and Burg expanding the number of cells before incorporating the cells in a scaffold for implanting. The resultant construct will be a cartilage construct as presently claimed. Smith et al disclose using a hydrostatic pressure and frequency of applying the pressure that are the same or substantially the same as used in the present claims. Perfusion with a medium as claimed during treatment with hydrostatic pressure would have been obvious to provide nutrients for the cells to maintain the cells active for growth. The conditions of dependent claims are suggested by conditions used by the references. Air contains slightly above 20% oxygen and using slightly less than 20%

oxygen would have been an obvious variation that would not be expected to produce a difference in result. Smith et al disclose 7.5% carbon dioxide (col 17, line 10), and using 5% as in claim 94 is an obvious variation that would not be expected to produce a difference in result. Atkinson et al further disclose repairing cartilage lesions, and if needed would have further suggested conditions that can be used.

Applicants disagree. Applicants maintain that the construct of the invention is different from the construct prepared by Smith et al and is not obvious in view of Lee, Burg and Atkinson.

Examiner summarily dismisses Applicants evidence and showing that the activated cells according to the invention under conditions as claimed have different properties and function better than cells activated under Smith protocol. The combination of four references to make the invention obvious makes no difference and does not make the invention more obvious just because these references somehow disclose various alternatives to the implant for treatment of cartilage.

It is true that when incorporating chondrocytes from cartilage into a scaffold for treatment as disclosed by Smith et al, it would have been obvious to expand the number of cells by *in vitro* culturing in a culture medium prior to incorporating the cells in the scaffold as suggested by Lee et al and Burg expanding the number of cells before incorporating the cells in a scaffold for implanting. However, the culturing and expanding cells is not the primary issue in this invention. The culturing and expansion of cells is only a preparatory stage for providing a sufficient number of cells to be available for incorporation into the support matrix having a spherically limited pore size so that a maximum number of cells gets incorporated into the matrix to provide for a functionally more efficient construct when these cells are activated.

The most important and primary aspect of the invention are conditions under which the activation of cells is performed. There Examiner is erring in not considering a unique combination of specific conditions under which such activation is performed. Such unique combination results in a construct that contains activated chondrocytes into a stage that is not achieved by simply using a hydrostatic treatment of Smith with or without a combination with Lee et al, Burg or Atkinson et al.

For example, Applicants clearly disclose finding of the importance of the limited size of pores. The limited pore size permits a faster infiltration of the chondrocytes into the matrix and faster growth and propagation of cells as well as higher density of the cells within the

construct. The limited pore size construct of the invention is able to be seeded with cell density between 3 and 60 millions/ml cells.

Applicants clearly show that the cells perfused with perfusion medium at a very low perfusion rate flow show much higher production of S-GAG and that, under exactly the same conditions, the rate of flow plays a role in such S-GAG production with a 10 times slower rate producing substantially and significantly more S-GAG (approx 30% more) than implants containing cells treated with 10 times faster flow rate. Par. [0187] clearly discloses that the higher extracellular accumulation is a result of a slower flow rate under which the activation is performed.

Applicants also clearly show that the reduced oxygen concentration plays a role in ability of chondrocytes to produce larger amounts of S-GAG. Examiner argues that “Air contains slightly above 20% oxygen and using slightly less than 20% oxygen would have been an obvious variation that would not be expected to produce a difference in result”.

Applicants respectfully suggest to the Examiner that the oxygen concentration between 2-5% **is not slightly lesser** but that it is 4-10 times lesser concentration than the atmospheric oxygen concentration used by Smith et al. As shown in Table 4, the GAG production at 20% oxygen concentration is less than half of the GAG production observed for 2%. That is not “slightly less” and cannot account for “obvious variation that would not be expected to produce a difference in result” as Examiner would have us believe.

Applicants cannot understand that Examiner would consider 4-10 times lower concentration of oxygen producing twice as much S-GAG as being obvious. Obvious from what? None of the references teaches importance of oxygen concentration. The same goes for the ten times lower perfusion flow. No reference teaches the importance or use of perfusion. How then it can be obvious to produce S-GAG from something that was not disclosed, implied, used or considered by any of the reference.

All these condition are performed at the same time during activation of cell in the tissue processor. Nowhere in any of the references is this or other conditions disclosed.

Applicants submit that the rejections under 35 USC 103 are overcome and should be withdrawn. Examiner is respectfully requested to do so.

SUMMARY

In summary, the application is refilled as RCE. Claims are amended to include new limitations and arguments are submitted to overcome the rejections under 35 USC 112, 102 and 103 forwarded by the Examiner in the prior Final Office Action. It is believed that all claims are in allowable conditions. The Notice of Allowance is respectfully requested.

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Respectfully submitted,



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